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Phenotypic fitness effects of the selfish B chromosome, paternal sex ratio (PSR) in the parasitic wasp *Nasonia vitripennis*

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Summary

B chromosomes are often considered genomic parasites. Paternal sex ratio (PSR) is an extreme example of a parasitic B chromosome in the parasitoid wasp *Nasonia vitripennis*. PSR is transmitted through the sperm of carrier males and destroys the other paternal chromosomes in early fertilized eggs. PSR disrupts the normal haplodiploid sex determination in this wasp by converting diploid (female) eggs into haploid (male) eggs that bear PSR. In this study I compare a number of phenotypic fitness aspects of PSR and standard (non-PSR) males. In general, PSR males were as fit as standard males. No significant differences were found in longevity (with one exception), ability to compete for mates and sperm depletion rates. PSR males produced 11–22% larger family sizes and developed slightly faster than standard males. Under conditions of sperm competition, females who mated with both types of males fertilized a constant proportion of eggs with each sperm type over their lifetime. PSR males produced fewer offspring among progenies from double-inseminated females. Phenotypic fitness effects are believed to play a minor role in determining PSR frequencies in natural populations.

Keywords: B chromosome, fitness, *Nasonia*, Paternal Sex Ratio, population genetics, selfish DNA, sperm competition

Introduction

Östergren (1945) originally described the parasitic nature of DNA in his studies of B chromosomes. However, only recently has the concept of 'selfish' or 'parasitic' DNA received wide attention (Dawkins, 1976; Nur, 1977; Doolittle and Sapienza, 1980; Orgel and Crick, 1980; Werren *et al.*, 1988; Bell and Burt, 1990; Shaw and Hewitt, 1990). Werren *et al.* (1988) defined selfish genetic elements as having characteristics that enhance their transmission relative to the rest of an individual's genome. These elements may be neutral or detrimental to the organism as a whole. Examples include certain repetitive DNA sequences, transposable elements, meiotic drive chromosomes, B chromosomes and non-Mendelian sex ratio distorters.

B chromosomes are chromosomes extra to the normal complement (A chromosomes) that vary in number between individuals of a species (Jones and Rees, 1982). They are considered genetically inert based on their small size and heterochromatic state. B chromosomes are transmitted at higher than Mendelian rates by either preferentially segregating into functional gametes during meiosis or by non-disjoining in mitosis (Jones, 1985). Generally, B chromosomes have no or only slight effects on their host fitness in low numbers, but in higher numbers they express increasingly harmful effects. From a parasitic point of view, B chromosomes are believed to be maintained in populations because their negative effects on host fitness are balanced by their replication drive.

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Detailed studies on the dynamics of B chromosomes are still very rare. Information on both transmission rates and fitness effects have only been obtained in four species: the lily *Lilium callosum* (Kimura and Kayano, 1961), the mealy-bug *Pseudococcus obscurus* (Nur, 1966a,b, 1969) and the grasshoppers *Melanoplus femurrubrum* (Nur, 1977) and *Myrmeleotettix maculatus* (Nur (1977) based on data of Hewitt (1973) and Robinson and Hewitt (1976)). These studies support the idea that B chromosomes reduce the mean fitness of individuals carrying them.

Paternal sex ratio (PSR) is an unusual B chromosome found in some natural populations of the parasitoid wasp *Nasonia vitripennis* (Werren *et al.*, 1987; Nur *et al.*, 1988; Werren, 1991). It is transmitted through the sperm of carrier males and destroys the paternal chromosomes (except itself) in the fertilized egg. Because this wasp has haplodiploid sex determination, the effect of PSR is to convert diploid eggs, which would normally develop into females, into haploid eggs that develop into PSR-bearing males. Thus, a female mated with a PSR male produces all-male offspring consisting of PSR males (fertilized eggs) and non-PSR males (unfertilized eggs). PSR gains a transmission advantage by haploidizing the genome with which it is associated, because unpaired chromosomes are transmitted at a low rate in meiosis (diploid females) but at a high rate in mitosis (haploid males) (Werren, 1991; Carlson and Roseman, 1992). Since PSR eliminates its host's genome each generation, it is an extreme example of a selfish genetic element (Werren *et al.*, 1988; Godfray and Harvey, 1989; Shaw and Hewitt, 1990).

PSR population dynamics have been studied in considerable detail. Werren (1987) and Skinner (1987) developed theoretical models for dynamics of PSR in panmictic populations. PSR dynamics in subdivided populations were investigated both theoretically (Werren and Beukeboom, 1993) and experimentally (Beukeboom and Werren, 1992). The sizes of local mating groups and the proportion of fertilized eggs were found to greatly affect the spread of PSR. These models of PSR dynamics have assumed that PSR males and non-PSR males have equal survival and mating success. However, there is little empirical data to support these assumptions. Here, I present experiments that measure the effects of PSR on the phenotypic fitness of its carrier. It should be realized that *Nasonia* males carrying the PSR chromosome have zero genetic fitness (i.e the classical fitness; Dawkins, (1982)), because they do not transmit any genetic material other than PSR to their offspring. However, the PSR chromosome may also have negative effects on the fecundity and survival of its carrier, which in turn may reduce PSR transmission. Therefore, phenotypic fitness effects have to be considered in the population dynamics of PSR.

Reproductive biology of Nasonia

The life history of *N. vitripennis* has been described earlier (Whiting, 1967; see also Werren, 1991; Beukeboom and Werren, 1992). Information on reproductive biology relevant to the fitness experiments is presented below.

Nasonia has haplodiploid sex determination. Males are haploid and develop from unfertilized eggs, whereas females are diploid and develop from fertilized eggs. As a consequence, unmated females can reproduce by laying unfertilized eggs, resulting in all-male progeny. Mated *Nasonia* females store sperm in a spermathecal organ. Normally, females have control over the sex ratio of their offspring by facultatively releasing sperm to their eggs upon oviposition (King, 1962). However, several sex ratio distorting elements have been found in natural populations of this wasp that prevent females from controlling the sex ratio of their offspring. In contrast to PSR, maternal sex ratio (MSR) distorts the sex ratio towards females. MSR is a maternally transmitted cytoplasmic element that causes females to fertilize 90–100% of their eggs (Skinner, 1982). The exact aetiology of MSR is unknown, but it probably interferes with spermathecal control.

Mating behaviour of *Nasonia* has been studied in detail (Barrass, 1960, 1961; Van den Assem *et al.*, 1980; Van den Assem, 1986). Males court in a frontal position on top of a female by

probing her antennae in bouts of head noddings and periodically releasing chemical stimulants. Immediately after a female signals receptivity by downsweeping her antennae, the male backs up and establishes genital contact. Copulation lasts approximately 20 s after which the male displays a short bout of post-copulatory courtship before he dismounts. Although single matings seem to be the rule in *Nasonia*, multiple matings can easily be induced in laboratory cultures. Sperm displacement is not known to occur in *Nasonia* (Holmes, 1974; Van den Assem and Visser, 1976).

A male's ability to inseminate females is limited, but the ability to induce receptivity in virgin females is practically unrestricted (Barras, 1961; Van den Assem and Visser, 1976). Neither the presence of sperm in the spermatheca, nor the acts of copulation are sufficient to switch off receptivity in females. Apparently, the onset of the receptive condition is the most important feature in this context. Post-copulatory courtship of males also serves to turn females off to second matings (Van den Assem and Visser, 1976). These authors conclude that natural selection has worked in *Nasonia* to prevent multiple insemination.

Material and methods

Standard culturing methods

Wasps were maintained on *Sarcophaga bullata* hosts. Generation time in *Nasonia* is approximately 14 days at 25°C. Virgin females and males were collected and isolated in the pupal stage by breaking open the *Sarcophaga* host at 11–13 days after egg laying. Adult wasps were usually one day old when used in experiments. Mated females were given hosts the day after mating for the first time, because they are unable to use sperm during the first hours after mating (Van den Assem and Feuth-de Bruijn, 1977).

Strains

The standard wild-type strain of *N. vitripennis* used in this study is Leiden LabII (subsequently referred to as LabII or 'Standard'). LabII females generally fertilize 80% of their eggs when ovipositing individually. The PSR chromosome is routinely maintained in a LabII background, designated as PSR(LabII) and is the same as used in previous studies (Werren and Van den Assem, 1986; Beukeboom and Werren, 1992, 1993).

In addition to the LabII strain, two high fertilization strains, MI (Macomb, Illinois; Saul *et al.*, 1965) and MIR5 (Rochester; New York; J. H. Werren, unpublished) were used. MI is the original strain carrying the MSR sex ratio distorter. MIR5 is a laboratory strain created by introgressing the MSR cytotype into the field-collected R5 strain. This was done to circumvent the high diapause tendency of the MI genotype. Females from these strains fertilize 90–100% of their eggs. The reason for using high fertilization strains was to reduce the proportion of non-PSR males (unfertilized eggs) among PSR progenies. This enhances the empirical comparison of PSR and standard progenies, because PSR is only transmitted to fertilized eggs.

The AsymC (asymbiont C) strain (Breeuwer and Werren, 1990) is a LabII strain cured of its microorganisms. Heritable microorganisms have been shown to cause incompatibility between certain strains of *N. vitripennis*. Incompatible crosses result in loss of the paternal chromosomes in fertilized eggs (Ryan and Saul, 1968; Breeuwer and Werren, 1990). Strains can be cured from their microorganisms with antibiotics. Males from the standard LabII (symbiont) strain are incompatible with females from the AsymC (asymbiont) strain.

Standard experimental procedures

Similar experimental procedures were used to compare various phenotypic fitness aspects of PSR and standard males. To eliminate strain-specific effects, PSR-carrying males with either the LabII

or MIR5 genetic background were always compared with non-carrier (standard) males from the same strain. A molecular assay (dot-blot; Beukeboom and Werren, 1992) was used to confirm the presence of PSR in all experimental males.

Male size in parasitoid wasps positively correlates with generation time (i.e. Van den Assem *et al.*, 1989), longevity (i.e. Charnov *et al.*, 1981; Van den Assem *et al.*, 1989; reviewed in Hurlbutt King (1987)), mating and insemination ability (Van den Assem and Visser, 1976; Jones 1982; Van den Assem *et al.*, 1989) and may affect sperm production, but this has not yet been investigated in any parasitoid (Hurlbutt King, 1988). Therefore, all comparisons between PSR and standard males utilized individuals of approximately equal size. In accordance with previous studies, head width was used as a standard measure of overall size. Head sizes were measured with an ocular micrometer under a stereo microscope (1 unit = 0.024 mm at × 40 magnification). Because adult size is often positively correlated with fecundity in females (e.g. Velthuis *et al.*, 1965; Charnov *et al.*, 1981; Lawrence, 1981; Waage and Ng, 1984; Liu, 1985; Tagaki, 1985; Opp and Luck, 1986; Mackauer and Kambhampati, 1988), mates were randomly taken from stock cultures.

In all experiments, female mates were virgin and given hosts the day after they had been used in crosses. Because host size can affect family size and developmental time (e.g. Hurlbutt King, 1988; Sequeira and Mackauer, 1992), hosts were always randomly allocated to experimental groups. Progenies emerged 2 weeks later and were scored for size and sex ratio. The presence of PSR among progenies was determined by pooling and dot-blotting five sons. This minimizes the chance of missing PSR because proportions of fertilized eggs (and, hence, PSR among progeny) were generally approximately 75% or higher and transmission of PSR to daughters is absent or very rare (Beukeboom and Werren, 1993).

Statistics

Differences between experimental groups were tested with the Mann–Whitney *U*-test using a 5% level of significance. In all cases, no difference between the experimental groups was used as the null hypothesis. For non-significant results the type II error, accepting the null hypothesis when it is false, has to be considered. I estimated the smallest difference that could be detected with a power of 0.80 under the given experimental conditions. For this I used the approximation for the power of the Mann-Whitney *U*-test outlined by Lehmann (1975).

Fitness measurements of PSR males

A number of components of survival and reproduction were compared between PSR and standard (non-PSR) males.

Development time (DT)

To determine the effect of PSR on the rate of development, two experiments measured the development times of PSR and standard males. The first experiment (DT I) compared egg to adult developmental time of all-male families from virgin MIR5 females with all-male families from PSR(LabII)-mated MIR5 females. Virgin and mated females were hosted for 24 h to stimulate egg production. They were subsequently given one host twice for 6 h, which resulted in two experimental sets. Short oviposition periods minimized the variance in egg laying time and subsequent progeny age and produced submaximal family sizes per host that minimized food competition among larvae. The first set of hosts from each group was placed at 20°C and the second set at 25°C. From each host, time of first emergence was determined by checking hosts every 4 h for the presence of emerged adults.

PSR males not only differ from standard males in the presence of the PSR chromosome, but also because they are derived from fertilized eggs, whereas standard males develop from unfertilized eggs. Fertilized eggs may develop faster than unfertilized eggs, as was shown for the honeybee *Apis mellifera* (Harbo and Bolten, 1981) and may therefore cause faster development of PSR males. A second experiment (DT II) eliminated a possible fertilization effect on development time. The experimental design was the same as in the previous experiment, except that the standard progenies were now also derived from fertilized eggs. This was accomplished by using a cytoplasmic incompatible (CI) cross. LabII males mated with AsymC females resulted in all-male AsymC families, because the paternal (LabII) chromosomes are destroyed in fertilized eggs. (Note that the effect of cytoplasmic incompatibility is the same as PSR action, although the two phenomena are mechanistically unrelated). Compatible crosses between PSR(AsymC) males and AsymC females also resulted in all-male AsymC progeny, because PSR destroys the paternal chromosomes. Thus, such progeny only differed from the CI-derived AsymC progenies by the presence of PSR. Development times of both groups were again compared at 20 and 25°C.

In DT I, PSR families were larger than standard males at both temperatures, but the differences were not significant (Mann–Whitney *U*-test; Table 1A). Development time was negatively correlated with family size, but not significantly for either group or temperature (Spearman rank correlation test; Table 1A). Development times were 18 days plus 19 h (n=31) for PSR versus 19 days (n=31) for standard males at 20°C and 13 days plus 6 h (n=32) versus 13 days plus 13 h (n=33) at 25°C. Thus, PSR progenies emerged earlier than standard progenies at both temperatures (5 and 7 h at 20 and 25°C, respectively), although the difference is only significant at 25°C (Mann–Whitney *U*-test; Table 1A).

In DT II, development times were 19 days plus 10 h (n=27) for PSR versus 20 days plus 12 h (n=28) for standard males at 20°C and 13 days plus 23 h (n=27) versus 14 days plus 2 h (n=30) at 25°C (Table 1B). PSR males developed significantly faster at both temperatures (26 and 3 h at 20 and 25°C respectively) but the difference was much larger at 20°C. Development time was again negatively correlated with family size in both groups at both temperatures (Spearman rank correlation test; Table 2B). Head width and family size did not correlate consistently, nor did head width and development time. Thus, within a group, family size did have a significant effect on development time, but individual size did not. PSR family sizes were not significantly larger than standard at both temperatures. However, the sizes of individual wasps in PSR progenies (based on the mean of two males per progeny) were significantly larger than in standard progenies (Mann–Whitney *U*-test; Table 1B). Nevertheless, the differences in development time remained significant at 20°C but not at 25°C after taking differences in family size and individual size into account (ANOVA; Table 1B). Both experiments had a power of 0.80 to detect differences in developmental time of less than 2.0%.

Hatch rate

Another experiment tested for a fertilization effect on development by measuring hatch rates of fertilized and unfertilized eggs. Virgin, PSR-mated and standard-mated females that had previously been on hosts, were given one new host for 4 h. Hosts were largely covered up by placement in a foam plug. Only one side was exposed to the wasp for oviposition in order to easily locate her eggs upon removing the host puparium. Hosts were kept at 25°C and opened 29 h later to determine the proportion of hatched eggs. Typically, more than 90% of the eggs hatch within 36 h after egg laying (at 25°C).

No significant differences were found in numbers of eggs laid and hatched between eggs from virgin, PSR-mated and standard-mated LabII females at 29 h after laying. Overall proportions of hatched eggs were 0.52 (n=22), 0.58 (n=11) and 0.44 (n=27), respectively (Mann–Whitney *U*-

Table 1. Development times (a and b) of PSR and standard males and hatch rate of (c) eggs from unmated (V, virgin), PSR-mated (P) and standard-mated (S) females

		PSR	Standard	Mann–Whitney *U*-test	MDD	Analysis of variance
(a) Development time I						
At 20°C	Development time (h)	451±9	456±13	$z = -1.643$, $p = 0.100$	1.82	
	Family size	23.0 ± 8.9	19.5 ± 9.6	$z = 1.490$, $p = 0.136$	29.53	
		$n = 31$	$n = 31$			
Spearman	DT vs FS	$r = -0.077$, $p = 0.672$	$r = -0.269$, $p = 0.141$			
At 25°C	Development time (h)	318 ± 6	325 ± 7	$z = 3.919$, $p < 0.0001$	1.47	
	Family size	22.7 ± 6.7	20.3 ± 8.5	$z = -1.340$, $p = 0.181$	24.2	
		$n = 32$	$n = 33$			
Spearman	DT vs FS	$r_s = -0.339$, $p = 0.059$	$r_s = -0.274$, $p = 0.121$			
(b) Development time II						
At 20°C	Development time (h)	466 ± 7	492 ± 6	$z = 6.368$, $p < 0.0001$	1.09	$F = 101.444$, $p < 0.0001$
	Head width (units)	29.5 ± 0.6	28.9 ± 0.6	$z = -3.608$, $p = 0.003$	1.59	$F = 26.551$, $p < 0.0001$
	Family size	33.0 ± 7.1	24.9 ± 5.4	$z = -1.643$, $p = 0.100$	14.87	$F = 89.603$, $p < 0.0001$
		$n = 27$	$n = 28$			
Spearman	DT vs HW	$r_s = 0.319$, $p = 0.098$	$r_s = 0.089$, $p = 0.654$			
	DT vs FS	$r_s = -0.237$, $p = 0.217$	$r_s = -0.394$, $p = 0.045$			
	HW vs FS	$r_s = -0.073$, $p = 0.704$	$r_s = -0.211$, $p = 0.283$			
At 25°C	Development time (h)	335 ± 6	338 ± 5	$z = 2.417$, $p = 0.016$	1.26	$F = 2.555$, $p = 0.116$
	Head width (units)	29.0 ± 0.4	28.7 ± 0.4	$z = -2.609$, $p = 0.009$	1.06	$F = 0.324$, $p = 0.578$
	Family size	25.8 ± 7.9	23.6 ± 6.9	$z = -1.465$, $p = 0.143$	21.96	$F = 3.542$, $p = 0.065$
		$n = 27$	$n = 30$			
Spearman	DT vs HW	$r_s = -0.220$, $p = 0.237$	$r_s = -0.323$, $p = 0.100$			
	DT vs FS	$r_s = -0.345$, $p = 0.063$	$r_s = -0.138$, $p = 0.480$			
	HW vs FS	$r_s = 0.282$, $p = 0.129$	$r_s = 0.092$, $p = 0.639$			

	PSR	Standard	Virgin	Mann–Whitney *U*-test	MDD
(c) Hatch rate					
Number of eggs laid	7.3 ± 3.7	9.0 ± 5.1	9.1 ± 4.0	P vs S z = 0.987, p = 0.324	67.60
				P vs V z = 1.304, p = 0.192	57.46
				S vs V z = 0.617, p = 0.537	42.87
Number of eggs hatched	4.2 ± 2.2	3.9 ± 3.1	4.7 ± 4.0	P vs S z = −0.357, p = 0.721	71.16
				P vs V z = 0.403, p = 0.687	90.05
				S vs V z = 0.842, p = 0.400	75.26
Per cent of eggs hatched	57.5	43.8	51.5		
	n = 11	n = 27	n = 22		

Development time (DT) was measured as time (in h) from egg laying to first adult emergence from the host at 20 and 25°C in two experiments. Also measured were family sizes (FS) and mean head widths (HW) in micrometer units (1 unit = 0.024 mm). In the first experiment, standard males were derived from unfertilized eggs (virgin females), but in the second experiment from fertilized eggs (by cytoplasmic incompatibility, see Methods). Hatching rate was measured as the number of eggs that had hatched 29 h after egg laying at 25°C. Values are means ± SD, n = number of families. Within a group, correlations between development time, family size and head width are tested with Spearman rank-correlation test. Differences between PSR and standard males are tested with the Mann–Whitney *U*-test and an analysis of variance (ANOVA, d.f. = 1 in all cases). MDD is minimal detectable difference with 0.80 power expressed as per cent of the first mean.

Table 2. Longevity of PSR and standard males under different conditions

	PSR	Standard	Mann–Whitney *U*-test	MDD
(1)	228 ± 31 (33)	248 ± 29 (35)	$z = 2.660$ $p = 0.008$	9.21
(2)	179 ± 18 (29)	182 ± 22 (46)	$z = 0.383$ $p = 0.702$	7.86
(3)	515 ± 131 (34)	530 ± 142 (46)	$z = 0.971$ $p = 0.331$	17.40
(4)	122 ± 7 (35)	124 ± 8 (35)	$z = 1.208$ $p = 0.227$	4.25
(5) Group 1	104 ± 8 (20)	101 ± 8 (20)	$z = -1.340$ $p = 0.180$	7.06
Group 2	104 ± 8 (20)	106 ± 6 (20)	$z = 0.788$ $p = 0.430$	6.24

Males are tested individually or in groups, with or without mates and food at 20 and 25°C. (1) Individually, unmated, without food at 20°C, (2) individually, unmated, without food at 25°C, (3) individually, unmated, with food at 20°C, (4) individually, mated, without food at 25°C, (5) in groups, unmated, without food at 25°C. Longevity is given in h ± SD with sample size in parentheses. Differences between PSR and standard males are tested with the Mann–Whitney *U*-test. MDD is minimal detectable difference with 0.80 power expressed as per cent of the first mean.

test, Table 1C). Recall that virgin females produce only unfertilized eggs that develop into standard males, PSR-mated females produce mostly fertilized eggs that develop into PSR males and standard-mated females produce mostly fertilized eggs that develop into females. Mated LabII females also lay some (approximately 20%) unfertilized eggs that develop into standard males. Although only large differences could be detected with reasonable power due to small sample sizes, the results suggest that the shorter development time of PSR males is not due to earlier hatching of PSR fertilized eggs.

Longevity

Adult lifespans of PSR and standard males were compared under the following conditions: (1) individually unmated at 20°C, without food, (2) individually unmated at 25°C, without food, (3) individually unmated at 25°C, fed sugar water every day, (4) individually mated with three LabII females at 25°C, without food and (5) in two groups of approximately 20 PSR and 20 standard males at 25°C, without food. All males were individually isolated into standard vials in the late pupal stage and grouped by equal age and size. Survival of wasps was measured at regular intervals: every 12 h in group 1, every 6 h in group 2, every 24 h in group 3 and every 4 h in groups 4 and 5.

The lowest survival rate occurred in groups at 25°C (approximate lifespan of 4 days and 8 h), and the highest when males were kept individually and fed sugar water daily (approximately 22 days, Table 2). The lifespans of PSR males were equal to standard males under all conditions, except when kept individually at 20°C without food and mates. Under such conditions PSR males lived almost one day less than standard males (PSR males 9 days plus 12 h versus standard males 10 days plus 8 h; Mann–Whitney *U*-test, $p < 0.01$; Table 2). The minimal differences between both groups that could have been detected with a power of 0.80 varied in the range 4–18%.

Family size

To determine whether family sizes from PSR and standard males were different, 88 PSR and 89 standard males were individually mated with MI females. Family sizes were routinely scored 2 weeks later. Family sizes of standard, PSR and virgin MIR5 families were also compared in the sperm depletion experiment (see below).

In the first experiment, PSR families were 11.2% (3.7 wasps per host) larger than standard families and in the other experiment 22.4% larger (8.9 wasps per host) in set I and 21.2% larger (7.6 wasps per host) in set II. All differences are significant (Mann–Whitney *U*-test; Table 3).

Table 3. Sizes of PSR (P), standard (S) and virgin (V) families are compared in two experiments

	PSR	Standard	Virgin	Mann–Whitney *U*-test	MDD
Experiment I	36.9 ± 1.3 (88)	33.2 ± 1.1 (89)	–	P vs S $d = +11.2\%$ $z = 2.281$, $p = 0.023$	1.41
Experiment II					
Set 1	48.5 ± 1.1 (84)	39.6 ± 1.0 (83)	46.6 ± 0.6 (278)	P vs S $d = +22.4\%$ $z = -5.756$, $p < 0.0001$	0.96
				P vs V $d = +4.1\%$ $z = -1.838$, $p = 0.066$	0.55
				S vs V $d = -17.6\%$ $z = 5.871$, $p < 0.400$	0.65
Set 2	43.6 ± 1.2 (49)	36.0 ± 1.2 (51)	39.4 ± 0.8 (139)	P vs S $d = +21.2\%$ $z = -5.152$, $p < 0.0001$	1.59
				P vs V $d = +10.7\%$ $z = -2.576$, $p = 0.010$	1.01
				S vs V $d = -9.5\%$ $z = 2.434$, $p = 0.015$	1.21

Values are means ± SD with sample size in parentheses. Differences (d) are tested with the Mann–Whitney U-test. MDD is minimal detectable difference with 0.80 power expressed as per cent of the first mean.

These results are consistent with family sizes in the development time experiments (see Table 1). In the second experiment, PSR families were also larger than those from virgin females (4.1% and 10.7% in sets I and II, respectively), although the differences are smaller than compared to standard families and only significant in set II. Standard families were significantly smaller than families from virgin females (17.6% and 9.5% in sets I and II, respectively). Recall that PSR progenies are all-male and standard progenies are female-biased. Therefore, part of the size difference between the standard and PSR families may be the result of the latter being all-male. A likely explanation is enhanced survival due to reduced food competition in all-male broods, because males are smaller and require less food for complete development than females. The difference in size between all-male families from PSR-mated and virgin females may be due to smaller clutches laid by unmated females or to higher survival from egg to adult of PSR males.

Mate competition (MC)

Two experiments tested whether PSR and standard males were equally competitive in obtaining mates. In experiment MC I, 22 pairs of one PSR and one standard male were presented with 20 LabII females for 4 h. The numbers of mates acquired by each male were determined from the sex ratio and presence of PSR in progenies. A second experiment (MC II) had the same design, but used competing males with different eye colours (wild-type and red) leading to daughter offspring with eye colours that were discriminative for father type. A LabII strain carrying the red eye colour recessive mutation st318 (Saul *et al.*, 1965) was used. Twelve pairs of one PSR(LabII^{st318}) and one standard (LabII^{wt}) male (group A), as well as 12 pairs of one PSR(LabII^{wt}) and one standard (LabII^{st318}) male (group B), were given 20 homozygous LabII^{st318} females for 2 h. The purpose of this experiment was to separate double matings (PSR and standard) from single matings with PSR males that incompletely transmitted the chromosome. Double matings result in daughters with the standard male's eye colour, but incomplete PSR transmission in daughters with the PSR male's eye colour. Three pairs of one LabII^{wt} and one LabII^{st318} male $\times$ 20 homozygous LabII^{st318} females (group C) served as control for a possible effect of the st318 mutation upon a male's competitive pairing ability.

Progenies were grouped according to sex ratio and the presence or absence of PSR and attributed to standard, PSR or double-mated females as explained in Table 4A. (1) Progeny with 'normal' female-biased sex ratios ($\leq$ 25% male) and absence of PSR were considered offspring of females sired by standard males. Because a small fraction (up to 5%) of PSR males sometimes completely fail to transmit PSR (Beukeboom and Werren, 1993), some of these progenies may actually have been from females sired by non-transmitting PSR males. This was neglected in the statistical analysis. (2) All-male progeny with the presence of PSR were the result of females sired by PSR males. (3) All-male progeny without PSR were the result of virgin females and were not attributed to either male. (4) Progeny with female-biased or intermediate (26–99% male) sex ratios and the presence of PSR among males could be offspring of a female that mated more than once (PSR and a standard male) and used both males' sperm. Alternatively, they could result from a female mated once with an incomplete transmitting PSR male. In addition to PSR males that produce 'normal' female-biased sex ratio progeny and do not transmit the chromosome at all, occasionally some males (up to 5%) produce various proportions of daughters and transmit the chromosome to their sons only (Beukeboom and Werren, 1993).

In MC I, a mean of 15.3 females of each competing group of 20 produced offspring (Table 5A). Among those, a mean of 1.0 had remained virgin, 6.5 had been mated by a standard male, 6.2 by a PSR male and 1.5 females had either been inseminated at least twice (standard and a PSR male) or once by an incomplete transmitting PSR male. Two statistical analyses were performed. One (conservative method) considered all progenies in the last category to be the result of multiple

Table 4. Progeny categories in mate competition experiments distinguished by the sex ratio, presence or absence of PSR in sons and the eye colour of daughters (experiment II only)

Type of mating and male genotype	Progeny sex ratio	PSR in sons	Eye colour of daughters
Mate competition I (female, LabII^{wt}; males, standard (LabII^{wt}) and PSR (LabII^{wt}))			
No mating	All-male	No	–
Single mating with			
Standard	Female-biased	No	Wild-type
PSR (complete transmission)	All-male	Yes	–
PSR (incomplete transmission)	Female-biased or intermediate	Yes	Wild-type
Double mating with			
Standard and PSR (complete transmission)	Female-biased or intermediate	Yes	Wild-type
Standard and PSR (incomplete transmission)	Female-biased or intermediate	Yes	Wild-type
Mate competition II group A (female, LabII^{st318,st318}; males, standard (LabII^{wt}) and PSR (LabII^{st318}))			
No mating	All-male	No	–
Single mating with			
Standard	Female-biased	No	Wild-type
PSR (complete transmission)	All-male	Yes	–
PSR (incomplete transmission)	Female-biased or intermediate	Yes	Red
Double mating with			
Standard and PSR (complete transmission)	Female-biased or intermediate	Yes	Wild-type
Standard and PSR (incomplete transmission)	Female-biased or intermediate	Yes	Wild-type and red
Mate competition group B (female, LabII^{st318,st318}; Males, standard (LabII^{st318}) and PSR (LabII^{wt}))			
No mating	All-male	No	–
Single mating with			
Standard	Female-biased	No	Red
PSR (complete transmission)	All-male	Yes	–
PSR (incomplete transmission)	Female-biased or intermediate	Yes	Wild-type
Double mating with			
Standard and PSR (complete transmission)	Female-biased or intermediate	Yes	Red
Standard and PSR (incomplete transmission)	Female-biased or intermediate	Yes	Red and wild-type

Female-biased sex ratios are the LabII standard sex ratios of ≤25% males and intermediate sex ratios are 26–99% males. Eye colour of daughters is either wild-type (wt) or red (st318 mutation). A double mating assumes sperm use of both males.

matings. Each such progeny was counted as both a standard and a PSR mating. The other (non-conservative method) attributed all such progenies to PSR matings with incomplete PSR transmission. Both methods revealed no significant difference between the number of mates acquired by PSR and standard males (Wilcoxon-test: conservative method, $z=0.181$, $p=0.856$,

Table 5. Mating status of females in the mate competition experiments

Pair number	Mating status V	S	P	D	Total	Pair number	Mating status V	S	P	D	Total
Mate competition I (standard (LabII) versus PSR (LabII) male)											
1	1	7	9	0	17	12	2	6	6	2	16
2	1	6	7	2	16	13	0	6	5	4	15
3	3	7	9	0	19	14	1	8	5	2	16
4	1	14	2	1	18	15	1	5	6	4	16
5	3	8	3	0	14	16	0	3	9	3	15
6	1	8	5	1	15	17	2	6	5	1	14
7	0	3	6	3	12	18	1	4	5	3	13
8	1	5	8	2	16	19	0	7	5	0	12
9	1	6	9	0	16	20	0	7	6	3	16
10	1	6	6	1	14	21	1	7	7	1	16
11	0	8	6	0	14	22	1	6	8	1	16
							–	–	–	–	–
Total							22	143	137	34	336
Mean							1.0	6.5	6.2	1.5	15.3
SD							0.9	1.9	2.2	1.3	1.7
per cent							6.5	42.5	40.5	10.1	

Pair number	Group A (PSR (LabII^{st318}) vs standard (LabII^{wt})) Mating status V	S	P	D	Total	Group B (PSR (LabII^{wt}) vs standard (LabII^{st318})) Mating status V	S	P	D	Total
Mate competition II										
1	2	5	3	2	12	1	3	10	2	16
2	0	7	9	2	18	2	8	2	5	17
3	0	5	10	2	17	0	9	3	2	14
4	1	7	8	2	18	2	9	6	0	17
5	0	4	10	2	16	3	3	4	3	13
6	8	9	1	0	18	3	6	5	0	14
7	1	4	6	3	14	0	6	8	1	15
8	2	2	3	4	11	0	7	8	2	17
9	0	4	6	2	12	0	7	4	3	14
10	0	7	8	2	17	0	8	9	0	17
11	1	6	7	4	18	3	8	5	1	17
12	1	4	6	2	13	0	8	5	0	13
	–	–	–	–	–	–	–	–	–	–
Total	16	64	77	27	184	14	82	69	19	184
Mean	1.3	5.3	6.4	2.3	15.3	1.2	6.8	5.8	1.6	15.3
SD	2.2	1.9	2.9	1.1	2.7	1.3	2.0	2.5	1.6	1.7
Per cent	8.5	34.6	41.8	15.0		7.8	44.4	37.9	10.5	
Both groups										
Total						30	146	146	46	
Mean						1.3	6.1	6.1	1.9	15.3
SD						1.8	2.1	2.7	1.3	2.2
Per cent						8.5	39.9	39.9	12.4	

Table 5 continued

Pair number	Group C (standard ($LabII^{wt}$) vs standard ($LabII^{st318}$)) Mating status V	st318	wt	D	Total
1	0	9	5	1	15
2	0	8	7	1	16
3	0	9	7	2	18
	–	–	–	–	–
Total	0	26	19	4	49
Mean	0	8.7	6.3	1.3	16.3
SD	0	0.6	1.2	0.6	1.5
Per cent	0	53.4	38.7	8.0	

One competing pair of a PSR and a standard male were provided 20 virgin females for a limited time. Females remained either virgin (V), mated once to either the standard (S) or the PSR (P) male or mated to both the standard and PSR male (D). Females that did not produce offspring are omitted. Both competing males in experiment I had wild-type eyes. In group A of experiment II, the PSR male had wild-type (wt) and the standard male red (St318 mutant) eyes, whereas in group B it was the reverse. In group C both males were standard and had either wild-type (wt) or red (st318 mutant) eyes. They serve as controls for a mating bias towards eye-colour. Numbers of acquired mates by PSR and standard males are determined from the mating categories as explained in Table 4. SD, standard deviation.

$n=22$ pairs; non-conservative method, $z=1.894$, $p=0.058$, $n=22$ pairs). The overall proportion of females that remained unmated in this experiment was 6.5% and the proportion that mated more than once was 10.1% (conservative method).

In MC II, no daughter offspring were produced with the PSR father's eye colour by any competing pair (in group A, PSR progenies with red-eyed females; in group B, PSR progenies with wild-type females; see Table 4B). Therefore, PSR transmission was 100% in this experiment and all progenies with female-biased or intermediate sex ratios and PSR present among sons, were attributed to multiple matings with both the standard and the PSR male. A mean of 15.3 females of each competing group of 20 produced offspring. Among those, 1.3 remained virgin, 6.1 mated once with a standard male, 6.1 mated once with a PSR male and 1.9 mated more than once (groups A and B pooled). Thus, the numbers of acquired mates by PSR and standard males were very similar (Wilcoxon-test: $z=0.043$, $p=0.966$, $n=24$ pairs). Proportions of virgin females (8.5%) and double-mated females (12.4%) were very similar to experiment I. These proportions are consistent with observations from other workers on mate competition in *Nasonia* (Holmes, 1974; Werren and Van den Assem, 1986). Interestingly, homozygous $LabII^{st318}$ females had a non-significant preference for mating st318 rather than wild-type males in all groups, but did not distinguish between PSR and non-PSR males (groups A and B). In conclusion, PSR males were as competitive in obtaining mates as non-PSR males, under this experimental regime.

Sperm depletion

A sperm depletion experiment determined whether PSR and standard males differ in the rate at which they become depleted of sperm. Six PSR(LabII) and six standard (LabII) one day old males were individually mated in rapid succession with 45 MI females (designated as set I). The males were then kept without mates for four days and fed sugar water. Sperm replenishment was measured on day five by presenting the same males with 25 MI females in succession (designated set II). The experimental design was such that as soon as a male had copulated with a female and

Table 6. Rate of sperm depletion in PSR(LabII) and standard (LabII) males

	PSR	Standard	Mann–Whitney *U*-test	MDD
Set 1				
Time to copulate				
First 10 mates	13.2 ± 0.8	13.3 ± 1.4	$z = 0.423$ $p = 0.672$	14.87
First 25 mates	33.2 ± 2.6	31.8 ± 2.6	$z = -0.898$ $p = 0.369$	13.48
All 45 males	61.5 ± 4.4	58.8 ± 3.7	$z = -1.376$ $p = 0.169$	11.38
Time to inseminate				
First 10 mates	23.2 ± 18.6	15.5 ± 4.2	$z = -0.325$ $p = 0.745$	100
Number inseminated				
Among first 25 copulations	13.3 ± 3.7	14.8 ± 3.1	$z = 0.647$ $p = 0.517$	44.19
Total	16.2 ± 4.7	19.2 ± 3.3	$z = 0.887$ $p = 0.375$	43.16
Set 2				
Time to copulate				
First 7 mates	12.2 ± 1.7	11.0 ± 0.6	$z = -1.341$ $p = 0.180$	17.99
First 10 mates	16.3 ± 2.4	16.2 ± 0.8	$z = 0$ $p = 1.0$	18.90
All 25 mates	41.0 ± 4.8	39.2 ± 1.8	$z = -0.890$ $p = 0.373$	15.22
Time to inseminate				
First 7 mates	15.3 ± 5.4	13.3 ± 4.5	$z = -0.809$ $p = 0.418$	55.94
Number inseminated:				
Among first 15 copulations	8.7 ± 2.3	9.5 ± 1.5	$z = 0.978$ $p = 0.328$	38.43
Total	10.3 ± 3.0	11.2 ± 2.1	$z = 0.814$ $p = 0.416$	43.29

Sperm depletion is measured as the time (in min) required to copulate and effectively inseminate mates and as the number of effectively inseminated females. Numbers are means ± SD and sample sizes are six in all cases. Differences between PSR and standard males are tested with the Mann–Whitney *U*-test. MDD is minimal detectable difference with 0.80 power expressed as per cent of the first mean.

dismounted her, the female was removed from the vial with an aspirator and the next one introduced. Usually, the male copulated with the new female within 1 or 2 min, even with later females. Thus, the total number of mates was limited by supply rather than by the males' ability to induce receptivity. The start of each copulation was recorded with a digital timer. Females were afterwards hosted in the standard way. This experiment also obtained data on family sizes from PSR-mated, standard-mated and unmated females.

Table 6 contrasts a number of parameters of speed and success of matings from six pairs of standard and PSR males. First, both PSR and standard males did copulate with every supplied female at equal speed (45 females in approximately 60 min in set I and 25 females in approximately 40 min in set II). In neither set were significant differences found between the two types of male in any parameter of mating speed. Only a portion of the copulations led to successful inseminations. In set I, standard and PSR males successfully inseminated means of 19.2 and 16.2 females and in set II means of 11.2 and 10.3 females, respectively (non-significant, Mann–Whitney *U*-tests; Table 6). Clearly, males became depleted of sperm on day one (set I) and had partly replenished sperm at day five (set II). Furthermore, the results indicate that PSR males were slightly slower in inducing receptivity and less successful in inseminating (transferring sperm to) females in rapid succession compared to standard males. However, only large differences could be detected with the sample sizes used.

Sperm competition

Double-mated *Nasonia* females have been found to use sperm of both males (Holmes, 1974; Van den Assem and Feuth-De-Bruijn, 1977; Werren and Van den Assem, 1986), suggesting the possibility of competition between males through sperm. Observations by Holmes (1974) and Werren and Van den Assem (1986) that mutant sperm had an advantage under certain conditions gave further support to this idea. A sperm competition experiment was designed to measure whether the PSR chromosome had an effect on sperm 'vigour'. The term 'sperm competition' is used here in a narrow sense, i.e. it refers to intraspermathecal competition of two types of sperm (Page and Metcalf, 1982).

Normally, *Nasonia* females mate only once and are unreceptive after the first copulation. However, females can be manipulated to mate more than once by preventing post-copulatory courtship of the first mate (Van den Assem and Visser, 1976). Competitive ability of PSR and standard sperm to fertilize eggs was measured in females mated to both a PSR(LabII) and a standard (LabII) male. A MIR5 female was placed with the first male in a mating chamber (glass dish, diameter 30 mm) covered with a glass plate. Immediately after copulation, the male was brushed off the female's back to prevent post-copulatory courtship and the second male introduced. Almost every female mated again under this design. In total, 11 females mated first with a PSR male followed by a standard male (group 'PSR^{1st}, standard^{2nd}') and 11 mated first with a standard male followed by a PSR male (group 'standard^{1st}, PSR^{2nd}'). Double-mated females were given two hosts after 24 h and, subsequently, two new hosts every day until death. The purpose of serial hosting was to (1) determine the proportional use of each male's sperm over a female's lifetime and (2) to measure total numbers of fertilized eggs and, hence, ejaculate size of males.

All males used in double matings were mated a second time with another MIR5 female. Those females were serially hosted similarly to the double-mated females and served three control purposes. First, they were used to check for complete PSR transmission by the PSR males. Secondly, they measured sperm use (fertilization rate) in the absence of competing sperm from the other male. Third, they yielded total numbers of fertilized eggs (ejaculate sizes of males) to single inseminated females. All PSR males (n=22) transmitted the PSR chromosome completely; no daughters were produced by the single-mated control females.

Table 7 shows that double-mated females, single PSR-mated control females and single standard-mated control females had very similar lifespans (approximately 18 days). They did not significantly differ in the number of days on which fertilized eggs were produced (means of 14.5, 14.0 and 16.5 days, respectively). Total numbers of eggs produced ranged from 600 to 1400 (means of 989 for double mated females and 926 for single standard-mated females, respectively), of which 400–1000 were fertilized (means of 701 and 789, respectively.) Lifetime egg production, as well as the total number of eggs fertilized, did not differ between double-mated experimental and single standard-mated controls. Fertilization proportions were not measured for single PSR-mated control females, because it would have required dot-blotting very large numbers of individual offspring. Data for both groups of double-mated females (standard^{1st}, PSR^{2nd} and PSR^{1st}, standard^{2nd}) were very similar and therefore pooled in Table 7. Minimal differences between the groups that could have been detected with a power of 0.80 varied in the range 15–27%.

In both groups of double-mated females only five out of 11 (45%) fertilized eggs with sperm from both males during their lifetime. The other six (55%) used only the first male's sperm. None of the females used only sperm from the second male. Females using only sperm from the first male may either be doubly inseminated without using the second male's sperm or they may be

Table 7. Lifespan and egg production of females that mated with both a PSR and a standard male (D) and singly with a PSR (P) or a standard (S) male in the sperm competition experiment.

	PSR and standard	Mating status of female			
		Only PSR	Only standard	Mann–Whitney *U*-test	MDD
Lifespan	18.6 ± 4.1 (22)	17.3 ± 5.5 (22)	18.6 ± 3.4 (22)	D vs P $z = -0.626$, $p = 0.532$	22.79
				D vs S $z = -0.317$, $p = 0.751$	17.69
				P vs S $z = -0.049$, $p = 0.961$	23.09
Fertilization days	14.5 ± 4.2 (22)	14.0 ± 4.9 (22)	16.5 ± 3.5 (22)	D vs P $z = -0.376$, $p = 0.707$	27.50
				D vs S $z = 1.414$, $p = 0.157$	23.29
				P vs S $z = 1.883$, $p = 0.060$	26.57
Total number of eggs	989 ± 184 (22)	Not measured	929 ± 178 (22)	D vs S $z = -1.338$, $p = 0.181$	15.99
Total number of fertilized eggs	701 ± 189 (16)	Not measured	789 ± 108 (22)	D vs S $z = 1.035$, $p = 0.301$	20.04

Presented are lifespan (in days), total number of days that fertilized eggs were laid, lifetime egg production and total number of fertilized eggs produced. Numbers are means ± SD with sample sizes in parentheses. Differences between groups are tested with the Mann–Whitney *U*-test. MDD is minimal detectable difference with 0.80 power expressed as per cent of the first mean.

singly inseminated by only the first male. Single insemination seems more likely because many females (28 out of 66, all females pooled) produced high proportions of unfertilized eggs during one or a few days, followed by exclusively unfertilized eggs a few days prior to death, suggesting they had used up all received sperm at that time. In addition, some females (six out of 66) produced high proportions of unfertilized eggs one or a few days prior to death, suggesting they were becoming sperm depleted. Females that lay exclusively unfertilized eggs at the end of their life have no sperm left in their spermathecae (L.W. Beukeboom, unpublished). Nadel and Luck (1985) have reported similar observations. Thus, if it is assumed that all females had used up all or most available sperm before they died and that all sperm survived until used, then the total number of fertilized eggs laid is a good approximation of the ejaculate size of the male. Using this reasoning, doubly and singly inseminated females received equal total numbers of sperm.

Proportional use of each male's sperm by doubly inseminated females differed dramatically (Table 8). Four out of five standard^{1st}, PSR^{2nd} doubly inseminated females used predominantly the first males' (standard) sperm, whereas all five PSR^{1st}, Standard^{2nd} doubly inseminated females used predominantly the second males' (standard) sperm. Thus, nine out of ten doubly inseminated females fertilized more eggs with standard than with PSR sperm (Sign-test, $p = 0.011$).

Daily utilization of each sperm type by doubly inseminated females over their lifetime is shown in Fig. 1. Use of each sperm type is constant over a female's lifetime. With one exception, daily sperm use of each sperm type is proportional to lifetime use by each doubly inseminated female (Kolgomorov-Smirnov tests, one out of ten significant; Table 8), indicating complete mixing of sperm. Only Standard^{1st}, PSR^{2nd}- mated female no. 1 used PSR sperm significantly later than standard sperm as shown by the day number at which she had used 50% of the total amount of sperm received of each type.

The results from the sperm competition experiment can be summarized as follows. Most double-mated females used only the first male's sperm and none used sperm from only the second male. Most likely, only a portion of second copulations resulted in effective sperm transfer. PSR males mated previously inseminated females as frequently as standard males, but sired

Table 8. Utilization of PSR and standard sperm by doubly inseminated females over their lifetime. Females were either mated first with a standard male followed by a PSR male (standard^{1st}, PSR^{2nd}) or vice versa (PSR^{1st}, standard^{2nd})

Female number	Number FD	Total FE	Fertilized eggs PSR	Fertilized eggs Standard	50% sperm use PSR	50% sperm use Standard	Kolgomorov–Smirnov
Standard^{1st}, PSR^{2nd}							
1	21	797	0.087	0.913	12	7	DN = 0.667 $p < 0.001$
2	13	454	0.132	0.868	5	5	DN = 0.154 $p = 1.0$
3	15	625	0.437	0.563	7	5	DN = 0.200 $p = 1.0$
4	10	638	0.489	0.511	6	5	DN = 0.200 $p = 1.0$
5	16	826	0.973	0.027	7	7	DN = 0.188 $p = 1.0$
PSR^{1st}, standard^{2nd}							
1	13	846	0.008	0.992	6	6	DN = 0.231 $p = 0.100$
2	11	657	0.041	0.959	5	5	DN = 0.182 $p = 1.0$
3	9	647	0.080	0.920	4	4	DN = 0.111 $p = 1.0$
4	19	967	0.088	0.912	10	7	DN = 0.316 $p = 0.300$
5	8	389	0.188	0.812	3	5	DN = 0.125 $p = 1.0$

Presented are the number of days that fertilized eggs were laid (number FD, fertilization days), total number of eggs fertilized (total FE), proportion of eggs fertilized by PSR and standard sperm and the day at which 50% of the total amount of sperm of each type was utilized. Differences in utilization of PSR and standard sperm are tested with the Kolgomorov–Smirnov test.

significantly fewer of their offspring, even when they were the first mate. Daily usage of standard and PSR sperm by doubly inseminated females was proportional to the total lifetime use.

Discussion

PSR is an exceptional B chromosome because it gains its transmission drive by eliminating the paternal chromosomes in the fertilized egg each generation. Because of haplodiploidy, this results in all-male families. Previous studies (Beukeboom and Werren, 1992; Werren and Beukeboom, 1993) identified population substructure and the proportion of fertilized eggs as important parameters in PSR dynamics. Models assumed that there is no phenotypic fitness cost associated with carrying the PSR chromosome. If PSR-carrying males were less fit than non-PSR males, the expected equilibrium frequency of PSR should be lower than if the two kinds of males were equally fit.

This study measured phenotypic fitness effects of the paternal sex ratio chromosome on individual males. In general, PSR males were as fit as standard males. No significant differences were found in longevity (with one exception where standard males lived longer), competition for mates or sperm depletion. Fitness differences were found in (1) family size (PSR progenies were 11–22% larger), (2) developmental time (PSR males developed faster) and (3) sperm competition (PSR males sired fewer offspring of doubly inseminated females). It is worthwhile considering the significance of these findings.

PSR males were shown to develop faster than standard males and both family size and wasp size were ruled out as a cause. In addition, faster development time was not due to the fact that PSR males are derived from fertilized eggs. These results stand in contrast to retarded development and increased cell cycle time as negative fitness effects of other B chromosomes (reviewed in Jones and Rees, 1982). Faster development could be selectively advantageous for PSR. It is known that natural populations of *Nasonia* are subdivided into local groups, in which

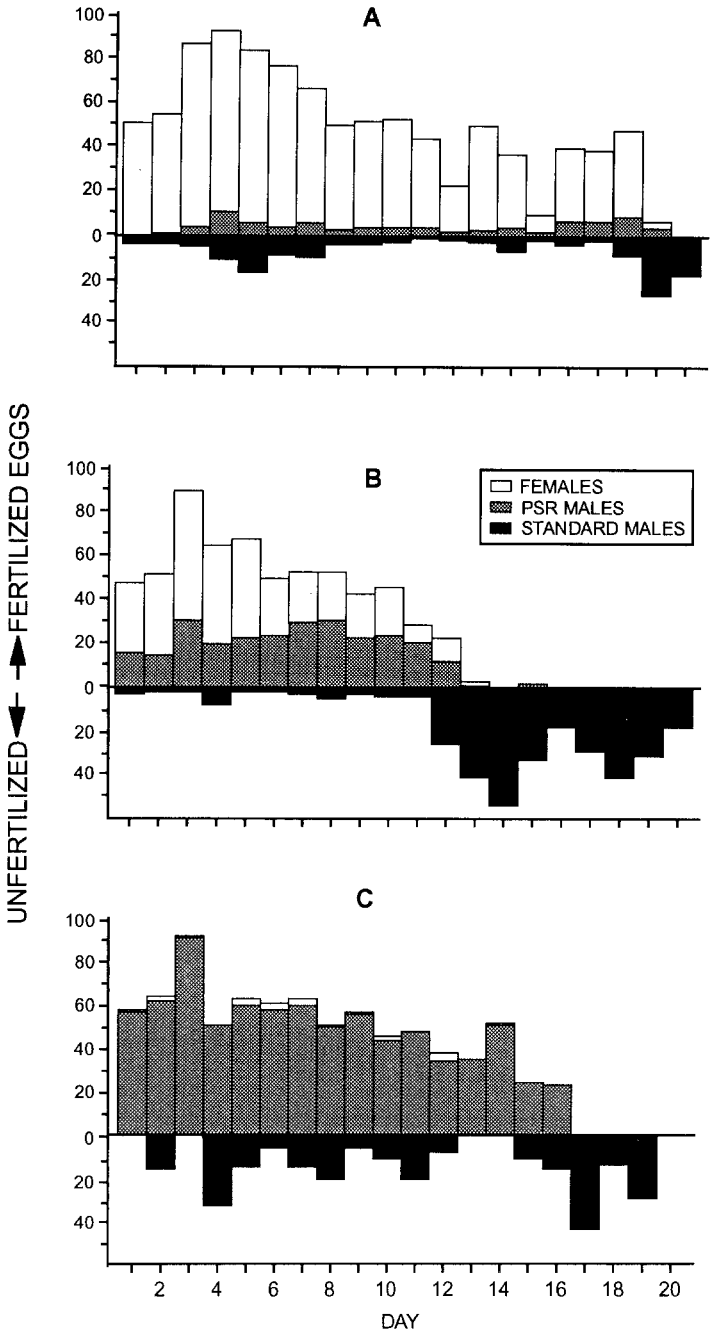

Figure 1. Daily utilization of PSR and standard sperm by doubly inseminated females in the sperm competition experiment. Included are females that utilized sperm from (A) predominantly the standard male ($N = 8$), (B) both types of males in equal proportions ($N = 1$) and (C) predominantly the PSR male ($N = 1$). Females start laying exclusively unfertilized eggs (standard males) towards the end of their life, which indicates that they become depleted of sperm.

offspring mate before females disperse in search of new hosts (Skinner, 1983; Werren, 1983). Normally, the flightless males emerge first and mate with females upon emergence. Under these conditions, by emerging early, males may acquire more mates. However, the precise gain in fitness by emerging early will depend on temporal and numerical variation in female emergences within a local mating group. In addition, both size (Van den Assem *et al.*, 1989) and fecundity (Van den Assem, 1976; Jones, 1982; Van den Assem *et al.*, 1989) are often positively correlated with development time in parasitoids. However, time to first offspring may be more important than increased fecundity as a function of size (Lewontin, 1965). In development time experiment II, PSR males were no smaller than standard males, even though they developed faster. This may be caused by the submaximal family sizes, whereas crowding may result in smaller individuals. Even so, PSR males probably gain more by developing faster than they lose by being smaller. The precise effects of faster development on fitness may be quantified empirically in laboratory and field population studies of *Nasonia*.

The adult lifespan of *Nasonia* was found to increase with supply of nutrients (Cousin, 1933) and decrease with mating (King and Hopkins, 1963). In addition, higher temperatures and interactions between males reduced longevity. PSR males lived as long as standard males, except that at 20°C without food they lived a slightly shorter time. Thus, in general, PSR did not significantly affect longevity.

PSR families were consistently 10–20% larger than families fathered by non-PSR males. This may be partially explained by reduced food competition among all-male broods (PSR) compared to female-biased broods (non-PSR), because all-male progenies from virgin females were also larger than broods from standard males, although not as large as PSR progenies. Therefore, the results also suggest that PSR males have increased survival rates from egg to adult, although mated females may lay more eggs than virgin females. These results contrast with *Spalangia cameroni* in which virgin and mated females produce equal families (Sandlan, 1979; Hurlbutt King, 1987). Male size was found to decrease with increasing family size. Because fecundity is positively correlated with size in parasitoid wasps (Van den Assem, 1976; Jones, 1982; Van den Assem *et al.*, 1989), the fitness advantage for PSR through larger family sizes may be partially cancelled out by smaller individual sizes.

The virility of PSR and standard males was generally equal. PSR males were equally successful as standard males in obtaining mates under conditions of direct competition. As in *Nasonia* (Werren and Van den Assem, 1986), sperm depletion has been reported from a number of parasitoid wasp species (Sekhar, 1957; Schlinger and Hall, 1960, 1961; Whiting, 1961; Wilson, 1961; Wiackowski, 1962; Wilkes, 1965; Gordh and DeBach, 1976; Nadel and Luck, 1985; Van den Assem *et al.*, 1989). Similarly to the present study, Barrass (1961), Gordh and DeBach (1976) and Nadel and Luck (1985) observed that males continued courting and copulating with females even when they no longer transmitted sperm. Results indicate that PSR males have somewhat reduced mating speed and sperm count.

Females in this study lived approximately 18 days and laid approximately 1000 eggs during their life. This value is similar to previously reported numbers (Velthuis *et al.*, 1965). Lifetime sperm production has not been reported for *Nasonia*. Although this was not precisely measured, a minimum estimate can be obtained from the results. In the sperm depletion experiment, males were shown to successfully inseminate at least 30 females (consistent with Barrass (1961) and Werren and Van den Assem (1986)) and probably more, but sperm replenishment was not measured after the second series of matings. The average female fertilized approximately 750 eggs. The pattern at which ejaculate sizes decreased in successive matings is unknown, but a linear function of sperm depletion, as was shown for *Pachycrepoideus vindemiae* (Nadel and

Luck, 1985), may be a good approximation. Combining these two values yields an estimated 11 250 (30 females × 375 sperm) sperm as a minimum estimate for lifetime sperm production in *Nasonia* males. This is far more than reported for any other parasitoid (see Gordh and DeBach, 1976), but two orders of magnitude smaller than the honey bee (Woyke, 1964).

Sperm use following multiple mating has been reported from three parasitoids, *Dahlbomius fuscipennis* (Wilkes, 1966), *N. vitripennis* (Holmes, 1974; Van den Assem and Feuth-de Bruijn, 1977; Werren and van den Assem, 1986) and *Lariophagus distinguendus* (Van den Assem *et al.*, 1989). Females from all three species typically mate only once, although *L. distinguendus* females may become receptive again later in life. Consistent with these authors, sperm mixing and a bias towards the use of sperm of the first male (when that male was standard (non-PSR)) was found. Unlike *D. fuscipennis*, additional matings in *N. vitripennis* apparently did not result in extra sperm transfer to the female's spermatheca. Crozier and Bruckner (1981) have suggested that the spermatheca of *Nasonia* may be too small to hold the ejaculates of more than one male. Even though the author's data and those of Holmes (1974) show that *Nasonia* females are able to store sperm from more than one male, an upper limit in sperm storage likely exists. Indeed, the finding that doubly inseminated females do not lay more fertilized eggs than singly inseminated females supports this notion (Mann–Whitney *U*-test: $z=0.488$, $p=0.625$, $n=10$ and 6, respectively; data not shown). This study found daily use of PSR and standard sperm to be proportional to lifetime use, but this has not been measured by other authors.

PSR and standard males were found to differ in one aspect of sperm competition. Doubly inseminated females fertilized more eggs with standard than with PSR sperm (four out of five and five out of five when the standard male was first and second mate, respectively). Although this may be expected when the standard male is the first mate of the two, it is surprising when the standard male is the second mate. There are several possible explanations. First, there may be competition in the spermatheca, i.e. the standard sperm may be more competitive than PSR sperm to fertilize eggs. However, if most females used up all sperm received, this explanation does not hold. Indeed, seven of the ten doubly inseminated females produced exclusively and two of them high proportions of unfertilized eggs at the end of their life. Moreover, the observed constant daily use of each sperm type in doubly inseminated females argues against this explanation.

A second explanation may be that standard males have larger ejaculate sizes than PSR males. Thus, ejaculates from PSR males may not completely fill the spermatheca, leaving space for additional sperm from the second (standard) male, whereas the reverse is not true. Nur (1966b) has shown reduced sperm count in mealy-bugs (*P. obscurus*) carrying B chromosomes. If the same is true in *Nasonia*, this should have been evident in the single control crosses. Unfortunately, logistic constraints prohibited the determination of total numbers of fertilized eggs (= ejaculate sizes) in the PSR control matings. However, the number of days that PSR-mated control females laid fertilized eggs (corrected for larger PSR families, analysis not shown) is not smaller than standard-mated control females. Therefore, it seems unlikely that PSR males transmit smaller ejaculates.

There is one more possible explanation for the observation that standard males are better second mates. They may sire more offspring of already inseminated females, not because of inherent larger ejaculate sizes, but because more standard sperm enters the spermatheca. The possibility that standard males are better sperm removers seems unlikely, because several authors concluded that sperm removal does not occur in *Nasonia* (Holmes, 1974; Van den Assem and Visser, 1976), and other hymenopterans (Taber, 1955; Wilkes, 1966). However, a more likely possibility is that standard sperm is faster in entering the spermatheca when competing with PSR

sperm. Because the second copulation took place immediately after the first, both types of sperm may have been simultaneously present in the vagina. Wilkes (1965) has shown for *D. fuscipennis* that sperm is ejaculated into a pocket formed in the posterior end of the vagina, from where it moves towards the spermathecal duct. Within a few minutes, 50% of the sperm had passed from there into the spermatheca and passage was complete within approximately 30 min. It is unknown, but not unlikely, that the same mechanism is operative in *Nasonia*. Standard sperm may then outcompete PSR sperm in entering the spermatheca until the maximum filling capacity has been reached and further entrance is prohibited. Considering this hypothesis, it would be interesting to know whether the double mated females that received sperm from only one male, copulated later for the second time than those that also received sperm from the second mate. Although all second matings took place within approximately 15 min after the first copulation, some variation occurred. Unfortunately, exact time intervals between first and second copulations were not recorded.

Important questions are whether observed differences in male virility and sperm vigour also occur under natural conditions and what their significance is for the population dynamics of PSR. First, multiple mating seems to be a phenomenon restricted to laboratory cultures of *Nasonia* (Van den Assem and Visser, 1976), although no data exist about frequencies of multiple mating in the field. Secondly, little is known about conditions for sperm competition under natural conditions. Sex ratios are generally female-biased and females disperse after mating. However, under specific conditions of population structure it may be possible for demic sex ratios to become male-biased (i.e. in the presence of PSR) and lead to intense competition for mates (Beukeboom and Werren, 1992). Conditions in the field may also be rare for males to become sperm depleted in that this requires large numbers of females (i.e. more than 20) per male at one time. Such strongly female-biased sex ratios may only occur in populations carrying the MSR element (Beukeboom and Werren, 1992). Again, field data are necessary to support this hypothesis.

Sampling throughout North America has so far detected PSR only in Utah, Idaho and Wyoming (J. Werren, unpublished). Here, PSR occurs in frequencies in the range 0–13% (Skinner, 1983; J. Werren, unpublished). Evidence indicates that natural populations of *Nasonia* are divided into temporary local mating groups (Skinner, 1983; Werren, 1983). However, not enough is known about the distribution and sizes of such demes (Beukeboom and Werren, 1992) to precisely evaluate their effects on the distribution and frequencies of PSR in nature. This study shows that the PSR chromosome has no substantial effects on the phenotypic fitness of its carrier, at least under laboratory conditions. PSR males are superior in some aspects of fitness (development time and family size), but inferior in others (sperm depletion and competition), which may counteract each other. Thus, unlike other reported B chromosomes (Kimura and Kayano, 1961; Nur, 1966a,b, 1969, 1977; Hewitt, 1973; Robinson and Hewitt 1976), phenotypic fitness effects are likely to play only a minor role in determining frequencies of PSR in natural populations.

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